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Browning of Sugar Solutions. VI. Isolation and Characterization of the Brown Pigment in Maple Sirup[a]

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SUMMARY

The colorant of maple sirup has been isolated using ion exchange resins and purified by dialysis. The purified pigment had an empirical formula of $C_{18}H_{27}O_{12}N$.

INTRODUCTION

The value of maple sirup resides strictly in its maple flavor. This attribute, however, cannot be measured objectively, and maple sirup is graded on its color. The lighter-colored sirups have less total flavor, but contain a more true, distinctive maple flavor than darker ones, and therefore command a higher market price. (The light-colored sirup is especially important in the making of maple confections.) Thus, the maple sirup producer must control the factors that cause the development of color in his product. Much excellent work has been done on the causes and control of color formation in sugar solutions and other food products. Hodge (1953), Liggett and Deitz (1954), and Zerban (1947) have published reviews in the field.

The color of maple sirup, as well as the flavor, is developed during the atmospheric boiling process used to concentrate maple sap to sirup. This was first demonstrated by Findlay and Snell (1935), who produced colorless sirup by vacuum-distilling maple sap, and was later confirmed at this laboratory by freeze drying. Also, the effect of pH on color development during the evaporation of sap was studied by Hayward and Pederson (1946) and Bois and Dugal (1940). Edson *et al.* (1912) observed that sap heavily contaminated with microorganisms produced dark-colored sirup. Hayward and Pederson (1946) noted that the invert sugar content of the sap affected the color of the sirup. Maple sap with no invert in it produces very light sirups. Holgate (1950) confirmed these findings. These observations correlate with data of Naghski and Willits *et al.* (1952) which also show that the sterile sap as it comes from the tree is fermented by adventitious organisms that produce different amounts of invert sugar. These reducing sugars are the main source of color in maple sirup. This information has permitted better control of the operations of sirup production, substantially increasing the amount of the lighter grades of maple sirup made. Nevertheless, knowledge of the mechanism of the color formation and of the chemical composition of the color bodies is still incomplete. Because the spectral characteristics of the color of maple products are similar to those of other food products and to sugar solutions, such information would be of value, then, not only to the maple industry but also to the sugar and food industry as a whole. Consequently, this study was initiated to determine the chemical composition of the colorant in maple sirups. Present information indicates that maple colorant is polymeric in nature, and further, in light-colored sirup may be totally different from that in dark-colored ones. This paper presents the results of work on the isolation and characterization of the colorant in light-colored sirups.

EXPERIMENTAL

Isolation of the Maple Sirup Colorant

Four hundred grams of Duolite A-4 anion exchange resin was washed free of suspended solids and packed as a wet slurry 78 cm deep in a glass column of 5.5×150 cm. A glass siphon tube

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was connected to the bottom of the column through a ball-and-socket joint. This served to maintain a constant level of solution 3 cm above the resin. Flow rate through the resin was controlled with a glass stopcock fitted to the top portion of the siphon tube.

The resin was put in the hydroxyl form by passing 18 L of 5% aqueous sodium hydroxide through the column. After the resin was washed free of excess alkali with distilled water, it was placed in the chloride form with 16 L of 5% sodium chloride solution. The resin was freed of excess chloride by washing again with distilled water.

A medium-amber grade of maple sirup, previously extracted with chloroform to remove maple flavor, was diluted with an equal volume of water and passed through the column. Eight liters of sirup saturated the resin with color, as indicated by the appearance of color in the effluent. At this point the flow of sirup through the column was stopped and the resin was washed free of sugar with distilled water.

The adsorbed maple color, free of sugar, was recovered from the column with 10 L of 5% sodium chloride. The elution with 5% sodium chloride was continued to regenerate the column as indicated by a clear eluate. Then the column was washed free of chloride and was ready for the passage of more sirup.

The brown maple colorant eluted from the ion exchange column was concentrated and freed of sodium chloride with a modification of the dialyzing concentrator described by Smith and Stevens (1942). A diagrammatic sketch of this unit is shown in Fig. 1. With a 250-ml. capacity, the

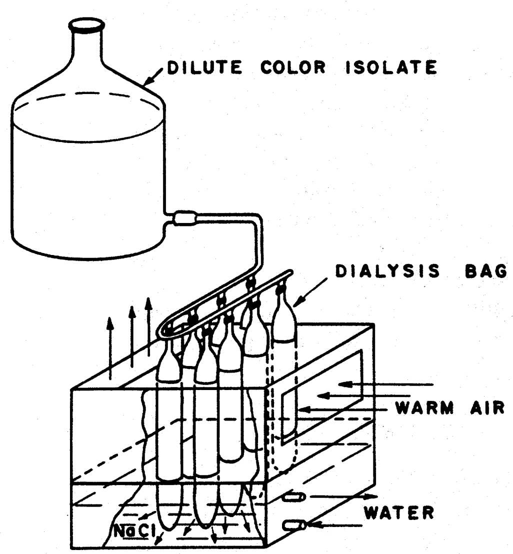

Fig. 1. Diagram of dialyzing concentrator.

apparatus evaporated water at the rate of 2 L per hour. The viscous dark-colored sirup obtained from 4 L of column effluent had a pH of 6.6. This color concentrate, when dewatered by lyophilization, yielded a dark-brown solid, which was further dried under vacuum for 4 hours (0.1 mm) at 40°C. This material proved to be high in ash, indicating that subsequent isolates should be further purified. Therefore, another color concentrate from the dialysis-evaporation unit was adjusted to a pH of 3 with hydrochloric acid and dialyzed against distilled water until free of chloride. The dried colorant from this additional treatment was low in ash. By this procedure 32 L of maple sirup gave 0.12 g (3–4 ppm) of dry colorant.

Characterization of the Isolated Pigment

1. *Elemental Analyses.* Two different samples of the isolated pigments were analyzed for carbon, hydrogen, nitrogen, and ash. The composition of the colorant from the dialysis concentrator before acidification was compared to that after acidification. The values are recorded in Table 1.

Table 1. Elemental composition of two samples of colorant from maple sirup.

	Sample [a]	
Determination	A [b]	B [c]
Carbon	48.5	47.8
Hydrogen	6.8	6.0
Nitrogen	3.6	3.1
Oxygen [d]	41.1	43.1
Ash	20.5	4.2

[a] Salt-free basis.
[b] From neutral salt solution.
[c] From acidified salt solution.
[d] By difference.

2. *Infrared Absorption Data.* Infrared absorption curves were obtained for the isolates from the neutral and acidified dialysis concentrates (Fig. 2). The pigments had characteristics of a carbohydrate, as indicated by strong hydroxyl and weak CH bands. The hydroxyl groups could be primary or secondary. No evidence of a carbonyl group was present in the neutral (unacidified) concentrate.

Strong absorption bands indicated the presence of carboxylate ion as an inorganic salt. Reduction of the ash by acidification and further dialysis weakened the carboxylate band. However, there was no evidence of a free carboxyl group building up, or of any other group that could form a salt. This is indirect evidence that the ash came from sodium salt of one of the organic acids, probably the malic acid present in the isolate as an impurity.

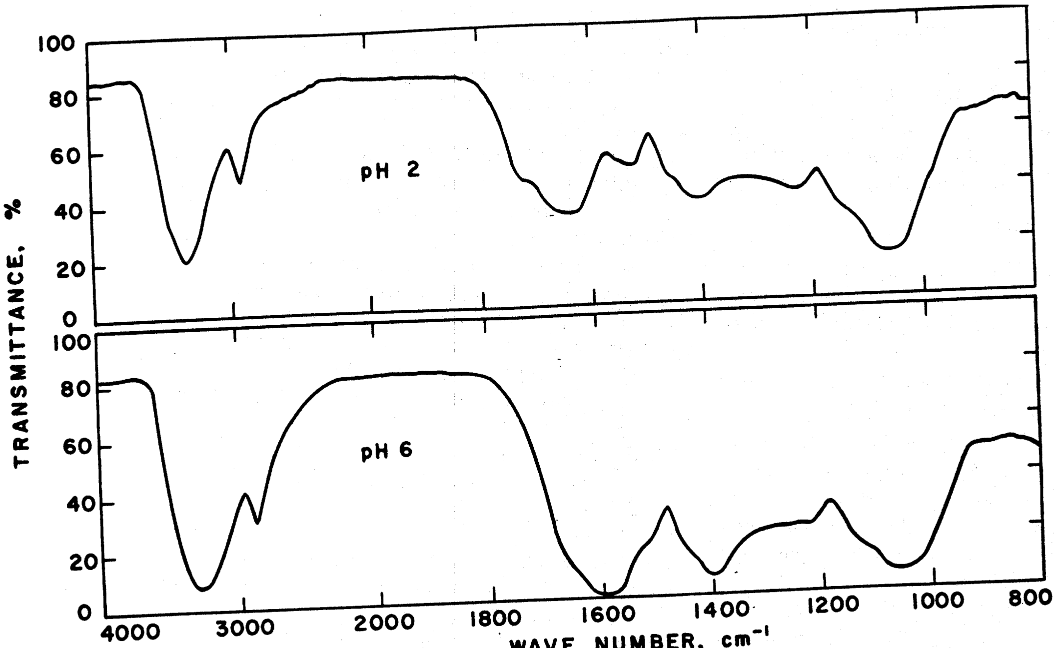

Fig. 2. Infrared curves of maple sirup colorant isolates before (pH 6) and after adjustment to pH 2 with acid.

3. *Ultraviolet Absorption.* The ultraviolet absorption curve at 400 to 225 mμ of the colorant from the neutral dialysis concentrate showed little character except a rapid increase in absorption as the wave length of light decreased, especially below 250 mμ. The acid treatment did not change this significantly.

DISCUSSION OF RESULTS

Several interesting properties of the colorant of maple sirup may be cited from the data:

1) Since the colorant can be separated from the sugars of the maple sirup with an anion exchange resin, it appears to be ionic and to carry a negative charge.

2) Elemental analysis indicates the colorant to have a composition similar to a carbohydrate containing nitrogen. The manner in which the nitrogen is incorporated in the colorant will be the subject of future investigations. Since the nitrogen content of maple sap is extremely small, about 10 ppm, it can be involved directly in forming only a limited amount of color. This would be more than enough to account for the small amount of colorant in a light-colored sirup (3–4 ppm isolated from the sirup used in this study). But maple sirup with continued boiling turns darker and darker. This latter color could be formed by a caramelization reaction, which may or may not involve nitrogen. Thus, two mechanisms could be responsible for the color of maple sirup.

3) The infrared absorption curves of the two colorants gave evidence that the pigment was carbohydrate in nature: absorption bands in the OH region were strong relative to the CH band.

4) Earlier work on isolating the colorant with ion exchange resins showed that the pigment is not homogeneous in composition. Maple sirup colorant that adsorbed on an anion exchange resin in the hydroxyl form could be separated into fractions by a series of increasingly alkaline eluting solutions—sodium chloride, sodium carbonate, and sodium hydroxide.

5) The maple colorant described here is quite similar to that isolated by W. W. Binkley, New York Sugar Trade Laboratory, by dialysis alone from cane final molasses (Binkley, 1957). From elemental analysis of the isolate he derived an empirical formula $C_{17}H_{26\text{-}27}O_{10}N$. The formula for the pigment in the maple sirup was found

by us to be $C_{18}H_{27}O_{12}N$. Thus, the compositions of the two colorants are very similar.

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